Mycoplasma genitalium: a cause of male urethritis?

Jørgen Skov Jensen, Randi Ørsum, Birthe Dohn, Søren Uldum, Anne-Marie Worm, Klaus Lind

Abstract

Background—Male urethritis may be caused by mycoplasmas. Since *Mycoplasma genitalium* has previously been isolated from the urethra of two men with non-gonococcal urethritis (NGU), it was the aim of the study further to elucidate its role by measuring the prevalence of this organism in men with NGU.

Material and methods—The polymerase chain reaction was used. Two different sequences of the gene coding for the main adhesin MgPa were amplified. Urethral, rectal, and throat samples from 99 male sexually transmitted disease (STD) patients with and without urethritis were studied.

Results—*M genitalium* DNA was demonstrated in 17/99 (17%) of the urethral swabs, but in none of the rectal and throat swabs. Significantly more patients with urethritis (13/52) were positive for *M genitalium* DNA than were patients without urethritis (4/47) (p < 0·03). In those with urethritis *M genitalium* DNA was found more often in *Chlamydia trachomatis* negative NGU (12/34) than in those with chlamydial NGU (1/14) (p = 0·05). Attempts to culture *M genitalium* from the PCR positive specimens were unsuccessful.

Conclusions—*M genitalium* DNA was found significantly more often in male STD patients with non-chlamydial NGU than in men with chlamydial urethritis (p = 0·05) and in men without urethritis (p = 0·003), suggesting that *M genitalium* may be a cause of NGU. *M genitalium* DNA was not demonstrated in any of the throat or rectal swabs indicating that the urogenital tract is probably the primary site of infection or colonisation of this species.

(*Genitourin Med* 1993;69:265–269)

Statens Seruminstitut,
Mycoplasma
Laboratory, Neisseria
Department,
Artillerivej 5, DK-2300
Copenhagen S,
Denmark
J S Jensen
B Dohn
S Uldum
K Lind

Department of
Dermatovenerology,
Bispebjerg Hospital,
Bispebjerg Bakke 23,
DK-2400 Copenhagen
NV, Denmark
R Ørsum
A-M Worm

Correspondence to
Dr J S Jensen

Accepted for Publication
28 April 1993

Introduction

C trachomatis is a well known cause of 30%–50% of non-gonococcal urethritis (NGU).[1] No other microorganism has been shown to cause any larger proportion of the remaining NGU cases. Treatment trials have indicated a role for *Ureaplasma urealyticum* in some 10% of NGU.[2] Ureaplasmas, however, have been isolated with the same high frequency from sexually transmitted disease (STD) patients with and without urethritis in many studies,[3 4] so the mere presence of ureaplasmas is not a sufficient indication of disease. The symptomatic effect of tetracyclines in the treatment of patients with urethritis from whom neither chlamydiae, nor ureaplasmas and other mycoplasmas could be isolated, led to the suspicion that uncultivable mycoplasma species could be the aetiology in a proportion of the unexplained cases of urethritis. In the search for such fastidious mycoplasmas *Mycoplasma genitalium* was isolated in 1981 by culture from two patients with NGU.[5] Despite several attempts this organism has, however, not been isolated from the urogenital tract since that time.

M genitalium has several properties in common with mycoplasmas known to cause disease, most prominently *M pneumoniae*: adherence to erythrocytes, epithelial cells, and plastic and glass surfaces; possession of a differentiated terminal structure which mediates adhesion; and ability to cause pathogenic changes in Vero cell cultures. Furthermore, animal studies have shown that *M genitalium* is able to colonise the male and female urogenital tract of non-human primates,[6-8] and to elicit a serological response. In some cases inflammatory reactions have also been described.

Isolation of *M genitalium* from clinical specimens is extremely difficult,[9] and diagnostic serology is hampered by extensive cross-reactions with the closely related *M pneumoniae*.[10 11] An attempt to evaluate the prevalence of *M genitalium* infections in men with urethritis with a DNA probe[12] has shown that the organism can be detected equally often in men with acute urethritis and in symptomless controls.

The recent observation that *M genitalium* could be found in respiratory tract specimens from patients with pneumonia and isolated in coculture with *M pneumoniae*[13] has raised new, interesting questions as to the main tissue tropism and the possible pathogenicity of *M genitalium*.

The main objective of this study has been to determine the prevalence of *M genitalium* in male STD patients with and without urethritis. For this purpose the polymerase chain reaction (PCR) technique[14 15] was applied.

Materials and methods

Patients and specimen collection

After giving informed consent 99 male patients from the outpatients' Venereal

Disease Clinic of Copenhagen were included; 52 attended the clinic because of urethritis and 47 attended the clinic for a STD check-up but without any symptoms of urethritis.

The patients with urethritis had dysuria and/or urethral discharge with ≥5 polymorphonuclear leucocytes per microscopic field (1000 × magnification) on a smear. Patients without clinical urethritis were not examined microscopically. None of the patients had received antibiotic treatment within one month before the examination.

Duration of symptoms, previous episodes of urethritis, number of sexual partners within the last month, sexual orientation, and presence or absence of respiratory tract complaints were recorded.

Each patient had three swabs taken from the urethra, the rectum, and the throat, respectively. The first swab placed in 2SP medium[15] was used for the culture of *C trachomatis*[16] and for the detection of *M genitalium* DNA by PCR; the second swab placed in Stuart's medium was for the culture of *Neisseria gonorrhoeae*[17] and *M hominis*[17] and *U urealyticum* in Shepard's 10C medium[18]; the third swab placed in SP4 medium[19] was for culture of *M genitalium* and for verification of PCR positive results obtained by analysis of specimens received in 2SP medium. The specimens intended for the culture of *M genitalium* were placed in liquid nitrogen within 12 hours and until use.

The results of all microbiological investigations were recorded without knowledge of the clinical data of the patients.

An acute phase serum sample was collected from all patients, but convalescent sera were not available.

M genitalium control strains
M genitalium G-37[T], was grown in SP4 medium and DNA extracted as previously described.[15] *M genitalium* strains M-30[5]; four throat isolates R 32 G, Tw 10–6 G, Tw 10–5 G, Tw 48–5 G,[13] and one synovial fluid isolate UTMB 10-G[20] were grown in SP4 medium and harvested by centrifugation; DNA was released by incubation in lysis buffer with proteinase K[15] and the crude lysate was used in PCR.

Procedure for culture of M genitalium from clinical specimens
From specimens positive for *M genitalium* DNA by PCR, 100 µl of the SP4 medium was inoculated into three different liquid media in tenfold dilutions of the original specimen up to 10^{-3}. The media were SP4 medium and two different modifications of the Friis' medium[18]; the latter two contained 78% of Friis' basal medium (64% Hanks' balanced salt solution; 0·4% (w/v) Brain-Heart Infusion (Difco, Detroit, MI, USA); 0·42% (w/v) PPLO broth without CV (Difco) in double-distilled (dd) water) supplemented with 5·8% of a 13·4% fresh yeast extract solution in dd water; 1% glucose; penicillin G 500 U/ml; and phenol red 0·002%. In one of the modifications the swine/horse serum mix-

ture was replaced by 14·3% heat treated (56°C/30 min) horse serum, in the other by an equivalent amount of fetal calf serum (Boehringer Mannheim GmbH, Mannheim, FRG). The final pH of both media was adjusted to 7·4. The inoculated broth media were incubated at 37°C and observed weekly for colour-change, and 50 µl was passaged onto solid media prepared with 0·5% agarose (HSA, Litex, Vallensbæk, Denmark). The plates were incubated at 36·5°C in an atmosphere of 8% O_2, 5% CO_2, and 87% N_2. The broth media were incubated for 12–14 weeks, but the agar media usually deteriorated after 4–6 weeks of incubation.

The M genitalium PCR assay
Primers were designed to amplify a 281 base-pair (bp) fragment of the main adhesin gene MgPa[21] using the same protocol for DNA-extraction and hybridisation as previously described.[15] Because the amplified region was found to show sequence variations, another set of primers amplifying a 453 bp fragment of the same gene (table 1) was chosen in order to verify and supplement the results. This PCR was optimised using dUTP instead of dTTP in all reactions allowing for enzymatic prevention of PCR product carry-over with uracil-N-glycosylase.[22] In brief, the reaction conditions for the second PCR assay were 1 × PCR buffer (50 mM KCl; 10 mM Tris hydrochloride [pH 8·3] at 25°C; 3·0 mM $MgCl_2$; 0·01% gelatin) containing 0·4 µM of each primer; 125 µM each of dATP, dGTP, and dCTP; 250 µM dUTP; and 2 U of *Taq* DNA polymerase (Amplitag; Perkin Elmer Cetus, Norwalk, Conn., USA). The samples were subjected to 50 cycles each consisting of 94°C/30s denaturation; 50°C/30s annealing and 72°C/1 min extension steps in a Hybaid Thermal reactor HBTR 1 (Hybaid Ltd., Teddington, Middlesex, UK).

Analytical sensitivity and specificity of the PCR assay
Both primer-combinations were able to detect *M genitalium* DNA equivalent to less than 10 cells in simulated positive clinical samples by visual inspection of ethidiumbromide stained agarose gels. All seven *M genitalium* strains tested reacted in the PCRs whereas DNAs from a wide variety of other mycoplasma species and bacteria known to be phylogenetically related to the mycoplasmas or to be common in the human microbial flora[15] did not react.

Precautions to avoid PCR product carry-over
Specimens to be tested in the *M genitalium* PCR were received, stored, and processed in a building separate from the PCR analysis laboratory. Dedicated micropipettors equipped with sterile filter tips (Aerosol Resistant Tips, SDS, Falkenberg, Sweden) were used in all manipulations with the samples. Polymerase chain reactions were assembled in a laminar air-flow hood by a technician who was not allowed to handle the amplified samples, and the recommendations of Ehrlich[23] were followed.

Table 1 Sequences of oligonucleotide primers used in PCR for verification

Name*	Sequence	Use
MgPa-476	5' ATG GCG AGC CTA TCT TTG ATC CTT TAA	Forward primer
MgPa-903	5' TTC ACC TCC CCA CTA CTG TCC TTA TGC	Reverse primer

*MgPa-476 corresponds to the sequence of bases 476 to 502 of the gene coding for the 140 kDa adhesin of *M genitalium*.[21] MgPa-903 is complementary to bases 903 to 929 of the same gene.

Serological investigations

Antigen for enzyme immunoassay (EIA) was prepared from *M genitalium* washed cell pellets lysed in 1% sodium dodecyl sulphate (SDS) and the EIA was performed essentially as described previously[24] except that immunoglobulin A (IgA) was detected in an additional assay with horseradish peroxidase conjugated rabbit anti human IgA (code P216 from DAKO, Copenhagen, Denmark).

Patients' sera were diluted 1:200 for detection of IgG and IgM antibodies and 1:50 for detection of IgA. Pools of sera from patients with respiratory tract infections showing high, medium and low reactions in our *M pneumoniae* complement fixation test[24] were used as standards.

Statistical analysis

Fisher's exact test (one sided) was used to test for differences in proportions, and the Mann-Whitney test was used to test for dissimilarity in distributions.

Results

The patients with and without urethritis were comparable with respect to median age, number of partners, and history of previous urethritis. The patients with urethritis included four with gonococcal, non-chlamydial urethritis, 14 with non-gonococcal chlamydial urethritis, and 34 with non-gonococcal non-chlamydial urethritis; 47 patients without urethritis served as controls (table 2).

M genitalium was detected by PCR in 17 (17%) of all 99 urethral swabs. 76% (13/17)

of the *M genitalium* positive specimens belonged to the group of patients with urethritis. As shown in table 2, *M genitalium* DNA was detected in 13/52 (25%) of all patients with urethritis while only 4/47 (8·5%) of those without urethritis were PCR positive (p < 0·03). The prevalence of *M genitalium* was 35% (12/34) in the group of men with chlamydia negative NGU. Compared with the patients without urethritis the difference in prevalence is highly significant (p = 0·003) but it is also higher than in those with chlamydia positive NGU (1/14) corresponding to 7% (p = 0·05).

M genitalium DNA could not be demonstrated in any of the throat or rectal swabs.

U urealyticum was isolated with the same frequency in men with urethritis as in men without in this study (table 2), even when the inoculum was quantified by 10-fold dilutions in broth (table 3).

M hominis was isolated equally seldom in patients with and without urethritis (table 2)

The number of homosexual men (n = 6) was too low to permit any calculations of the prevalence of *M genitalium* infection relative to sexual preference. There was no difference in the duration of symptoms, number of previous episodes of urethritis, and number of sexual partners within the last month between patients with gonococcal, chlamydial and non-chlamydial NGU. None of the patients had symptoms from the respiratory tract.

The two different PCR assays were comparable with respect to sensitivity as determined by the number of positives detected and with a complete concordance between the two primer-pairs. Some of the *M genitalium* positive specimens, however, showed marked differences in the amount of PCR product when the intensity of the ethidiumbromide stained bands from the same DNA extractions were compared. The region amplified with the MgPa-476/MgPa-903 primer-pair revealed a remarkable variation in the restriction fragment lengths (RFLs) between the amplicons

Table 2 Microorganisms recovered from urethral swabs of 99 male patients, 52 with urethritis and 47 asymptomatic cases examined at a sexually transmitted disease clinic. (Percentages in brackets)

	Number (%) of patients with urethritis				
Microorganism	All urethritis n = 52	Gonococcal n = 4	Chlamydial n = 14	Unspecified n = 34	Without urethritis n = 47
N gonorrhoeae	4 (8)	4 (100)	— —	—	0 (0)
C trachomatis	14 (27)	0 (0)	14 (100)	—	4 (9)
U urealyticum	16 (31)	0 (0)	4 (29)	12 (35)	22 (47)
M hominis	2 (4)	0 (0)	0 (0)	2 (6)	3 (6)
M genitalium*	13 (25)	0 (0)	1 (7)	12 (35)	4 (9)

*Detected by PCR only.

Table 3 Presence of Ureaplasma urealyticum in male patients with and without urethritis. (Percentages in brackets)

	Number (%) of patients				
Isolation of U urea-lyticum	All urethritis n = 52	Chlamydial urethritis n = 14	M genitalium PCR pos. urethitis n = 13	M gen.neg non-chlamydial NGU n = 21	Controls (no urethritis) n = 47
Negative	36 (69)	10 (71)	9 (69)	12 (57)	25 (53)
Positive	16 (31)	4 (29)	4 (31)	9 (43)	22 (47)
≥10³ ccu*	12 (23)	3 (21)	3 (23)	7 (33)	17 (36)
≥10⁴ ccu*	10 (19)	2 (14)	2 (15)	7 (33)	11 (23)

*Colour changing units (see Materials and Methods).

from different patients as compared to the identical pattern of RFLs obtained with the seven strains isolated by culture (data not shown).

M genitalium could not be cultivated from the PCR positive samples in any of the three different mycoplasma media.

Antibodies (IgM, G, and A) reacted in the EIA with *M genitalium* antigen at low levels both in patients with and without urethritis, regardless of their reaction in the *M genitalium* PCR. Two of the PCR positive patients had had complaints of urethritis for 30 days; one of them showed some IgM reactivity but it was not higher than that found in sera from some of the *M genitalium* PCR negative patients.

The reactivity of the pools of patients' sera selected as standards on the basis of reactions in the *M pneumoniae* complement fixation test showed parallel reactions in the EIAs with *M pneumoniae* and *M genitalium* antigens, although the reactivities with the *M genitalium* antigen were lower. This indicates a significant amount of cross-reactions.

Discussion

M genitalium DNA was demonstrated significantly more often in men with urethritis than in men without. This points to a possible role of this organism in NGU, but more studies are needed to establish its significance as a pathogen. We find it noteworthy that *M genitalium* was detected most often in men with non-chlamydial NGU. This may indicate that *C trachomatis* and *M genitalium* could act as separate causes of NGU.

The concept of causality has been modified several times since the Henle-Koch postulates were presented in 1884[25]; it has been particularly important to accept the existence of asymptomatic carriers. Taylor-Robinson has outlined these modified criteria that need to be fulfilled before regarding a mycoplasma as a cause of NGU.[26] The criteria are briefly: (i) mycoplasmas should be isolated more frequently from patients with disease than from those without; (ii) an antibody response should be demonstrated in patients with disease; (iii) the organisms should disappear and the disease respond to treatment with antimicrobial agents to which the organisms are susceptible *in vitro*; (iv) the organisms should be able to infect an animal host and produce disease similar to that seen in man; furthermore, they should be recovered during disease from the infected animal. To establish *M genitalium* as a cause of NGU the fulfilment of criteria (ii) and (iii) are needed. In this study we did not evaluate the response to treatment, neither did we collect convalescent sera. We are planning to do a new study to clarify these issues. Owing to the extensive serological cross-reactions between *M genitalium* and *M pneumoniae*,[11] a rise in antibody titre to either species during acute urethritis can only be presumptive for an *M genitalium* infection if the patient has no concomitant respiratory infection. In this study *M genital-*

ium was not detected in specimens from the throat or the rectum indicating that the urogenital tract is likely to be the primary site of infection/colonisation. In previous studies it has been suggested that *M genitalium* may reside in the gastrointestinal tract due to the higher prevalence of this organism in the urethra of homosexual men as determined by a whole-genomic DNA probe,[12] and the observation of a change in colour of the SP4-media inoculated with rectal swabs from homo- and heterosexual men.[9]

The failure to demonstrate *M genitalium* DNA in the throat swabs does not rule out the possibility that *M genitalium* may be a pathogen in the respiratory tract in patients with respiratory symptoms. It is remarkable that *M genitalium* has been isolated only in coculture with *M pneumoniae* from throat swabs in adults.[13] In this context it remains to be determined whether *M genitalium* could be acquired by the child from an infected mother during delivery and then become a cause of pneumonia or conjunctivitis in the newborn.

Despite quantitative culturing for *U urealyticum* we were unable to demonstrate any association between this organism and urethritis (table 3). This is not surprising since many studies have been unable to prove a significant role for *U urealyticum* in urethritis (reviewed by Taylor-Robinson[34]). The rare isolation of *M hominis* may reflect that the media used were not optimal for culture of this species.

Our cultural methods for isolation of *M genitalium* obviously need to be improved. A very important observation was made during our efforts to culture *M genitalium* from the PCR positive specimens. Although great care was exercised to avoid sample to sample contamination, one of our PCR positive specimens became infected with *M genitalium* G-37[T], probably from the cultures performed for quality control of the media. The contamination was disclosed only by the differences in restriction enzyme cleavage sites of the PCR product between the type-strain and the clinical specimen. As a consequence we now perform our further comparative studies of media in a laboratory in a separate building.

The concordance of the two PCR primer-pairs is encouraging in the sense that in theory the use of a single set of primers should be sufficient to detect all strains of *M genitalium*. It is obvious from restriction enzyme cleavage patterns of the amplicons produced from the clinical specimens, that *M genitalium* is a more heterogeneous species than previously assumed.[27] Marked differences between individual patients' strains were observed as opposed to the homogeneous pattern observed in the previously isolated strains.[15] Two of the patients harboured *M genitalium* strains which were so different in the region amplified with the MgPa-476/MgPa-903 primer pair, that an internal oligonucleotide probe did not hybridise. These two patients were indeed true PCR positive because another internal

probe hybridised and a third primer-set produced amplicons of the expected size.

The higher prevalence of *M genitalium* in patients with chlamydia negative NGU is not sufficient to prove that *M genitalium* can be a cause of NGU, though it is an important criterion. More knowledge about *M genitalium* may be gained by the use of PCR, also as a marker for the selection of specimens that should be further examined in future culture media and by new methods.

Data from this study have been presented as a poster at the 9th International Congress of the International Organization for Mycoplasmology held in Ames, Iowa, USA 2–7 August, 1992.

1 Bowie WR, Alexander ER, Holmes KK. Etiologies of postgonococcal urethritis in homosexual and heterosexual men: Roles of *Chlamydia trachomatis* and *Ureaplasma urealyticum. Sex Transm Dis* 1978;5:151–4.
2 Coufalik ED, Taylor-Robinson D, Csonka GW. Treatment of nongonococcal urethritis with rifampicin as a means of defining the role of Ureaplasma urealyticum. *Br J Venereal Dis* 1979;55:36–43.
3 Taylor-Robinson D, McCormack WM. The genital mycoplasmas (first of two parts). *N Engl J Med* 1980;302:1003–10.
4 Taylor-Robinson D, Tully JG, Furr PM, *et al.* Urogenital mycoplasma infections of man. A review with observations on a recently discovered mycoplasma. *Isr J Med Sci* 1981;17:524–30.
5 Tully JG, Taylor-Robinson D, Cole RM, Rose DL. A newly discovered mycoplasma in the human urogenital tract. *Lancet* 1981;I:1288–91.
6 Taylor-Robinson D, Furr PM. The pathogenicity of a newly discovered human mycoplasma (strain G37) for the genital tract of marmosets. *Journal of Hygiene (Cambridge)* 1982;89:449–55.
7 Taylor-Robinson D, Tully JG, Barile MF. Urethral infection in male chimpanzees produced experimentally by *Mycoplasma genitalium. Br J Exp Pathol* 1985;66:95–101.
8 Tully JG, Taylor-Robinson D, Rose DL, Furr PM, *et al.* Urogenital challenge of primate species with *Mycoplasma genitalium* and characteristics of infection induced in chimpanzees. *J Infect Dis* 1986;153:1046–54.
9 Taylor-Robinson D, Furr PM, Hanna NF. Microbiological and serological study of non-gonococcal urethritis with special reference to Mycoplasma genitalium. *Genitourin Med* 1985;61:319–24.
10 Lind K. Serological cross-reactions between *Mycoplasma genitalium* and *M pneumoniae. Lancet* 1982;ii:1158–9.
11 Lind K, Lindhardt BØ, Schütten HJ, Blom J, *et al.* Serological cross-reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae. J Clin Microbiol* 1984;20:1036–43.
12 Hooton TM, Roberts MC, Roberts PL, *et al.* Prevalence of *Mycoplasma genitalium* determined by DNA probe in men with urethritis. *Lancet* 1988;1:266–8.
13 Baseman JB, Dallo SF, Tully JG, Rose DL. Isolation and characterization of *Mycoplasma genitalium* strains from the human respiratory tract. *J Clin Microbiol* 1988;26:2266–9.
14 Saiki RK, Scharf S, Faloona F, *et al.* Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985;230:1350–4.
15 Jensen JS, Uldum SA, Søndergård-Andersen J, *et al.* Polymerase chain reaction for detection of *Mycoplasma genitalium* in clinical samples. *J Clin Microbiol* 1991;29:46–50.
16 Kristensen GB, Bollerup AC, Lind K, *et al.* Infection with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in women with acute salpingitis. *Genitourin Med* 1985; 61:179–84.
17 Lind K, Kristensen GB, Bollerup AC, *et al.* Importance of Mycoplasma hominis in acute salpingitis assessed by culture and serological tests. *Genitourin Med* 1985; 61:185–9.
18 Razin S, Tully JG, eds. *Methods in Mycoplasmology; Volume 1. Mycoplasma Characterization,* New York: Academic Press, Inc., 1983;
19 Tully JG, Whitcomb RF, Clark HF, Williamson DL. Pathogenic mycoplasmas: cultivation and vertebrate pathogenicity of a new spiroplasma. *Science* 1977; 195:892–4.
20 Tully JG, Baseman JB. Mycoplasma. *Lancet* 1991;337: 1296.
21 Dallo SF, Chavoya A, Su CJ, Baseman JB. DNA and protein sequence homologies between the adhesins of *Mycoplasma genitalium* and *Mycoplasma pneumoniae. Infect Immun* 1989;57:1059–65.
22 Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* 1990;93:125–8.
23 Ehrlich GD. Caveats of PCR. *Clin Microbiol Newslet* 1991;13:149–51.
24 Uldum SA, Jensen JS, Søndergård-Andersen J, Lind K. Enzyme immunoassay for detection of immunoglobulin M (IgM) and IgG antibodies to *Mycoplasma pneumoniae. J Clin Microbiol* 1992;30:1198–1204.
25 Evans AS. Causation and disease: The Henle-Koch postulates revisited. *Yale J Biol Med* 1976;49:175–95.
26 Taylor-Robinson D. The role of mycoplasmas in nongonococcal urethritis: a review. *Yale J Biol Med* 1983;56:537–43.
27 Razin S, Tully JG, Rose DL, Barile MF. DNA cleavage patterns as indicators of genotypic heterogeneity among strains of *Acholeplasma* and *Mycoplasma species. J Gen Microbiol* 1983;129:1935–44.